PB-227 485

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November 1959

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WP-151

WP-151 (C)

FINAL REPORT ON PROJECT K11 (C1)

September 1, 1957 to August 31, 1959

AEROBIC METABOLISM OF CYANOGENIC COMPOUNDS

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SUMMARY

The aim of this research was to obtain basic data on the aerobic metabolism of potassium cyanide. Studies were conducted on activated sludge cultures fed cyanide as their only source of carbon and nitrogen at hour and one-half hour intervals and continuously. It was shown that these cultures could metabolize the cyanide converting the carbon to carbon dioxide and the nitrogen to ammonia, nitrite, or nitrate. Little growth was realized in these systems although suspended solids could be maintained. Further studies showed that a cyanide acclimated culture readily metabolized a cyanide-acetate feed with the acetate producing considerable growth in the system. This latter culture removed 95 percent of the feed C.O.D. and converted the cyanide nitrogen to cell nitrogen and soluble inorganic nitrogen, ammonia, nitrites, or nitrates.

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I. INTRODUCTION

Cyanide wastes produced in the metal plating and the metal-hardening industries are said to be the most toxic wastes produced in industry. Cyanides are also found in the wastes from the manufacture of chemicals, plastics, coke and gas, refining of gold and silver, and the scrubbing of blast furnaces. These wastes contain both organic and inorganic cyanides ranging in concentration from 10 mg/l to as high as 30,000 mg/l CN^-.

The toxicity of cyanide is affected by a variety of factors. It is related to the degree to which the compound dissociates to yield the cyanide ion, the pH of the solution, the chemical composition of the toxic materials, and the synergistic effects of the other constituents in the wastes.

Stream conditions are definitely affected by minute quantities of cyanide. Renn (17) found that a potassium cyanide concentration of 0.07 mg/l CN^- with a dissolved oxygen content of 5.0 mg/l was very toxic to fish life. An indirect effect of the cyanide ion may be the killing of organisms which ordinarily live in a stream and assimilate limited amounts of organic matter from other sources of pollution, thus keeping the water in a reasonably pure state. The killing of these organisms interrupts the self-purification process of the stream and the water may become so polluted as to be impossible to treat by any of the processes normally used at a water treatment plant. The maximum

cyanide concentration allowed in streams by most states is less than 1.0 mg/l CN^-; however, some states allow no cyanide to be discharged into their streams.

Cyanide has been found to have various effects upon sewage treatment. Lockett and Griffiths (9) found that 4.0 mg/l CN^- had a definite inhibitory effect upon fermentation in anaerobic digestion. Ridenour, Backus, and Sherron (18) found that 1.6 mg/l HCN retarded digestion, although digesters could be acclimatized up to 16.5 mg/l HCN. Lockett and Griffiths (9) and Ridenour and Greenbank (19) found an appreciable decrease in nitrification in the activated sludge process due to 2.0 mg/l HCN. Pettet (13) found that concentrations of 1.0 mg/l HCN interfered with trickling filter operation.

Most of the present day cyanide disposal methods are chemical processes. A few of the methods are: chlorination (by gas, calcium hypochlorite, and chlorine dioxide), acidification, and electrolytic oxidation. Chlorination is expensive due to the required mixing, sludge disposal, and the chemicals required to adjust the pH. Electrolytic oxidation and acidification produce toxic hydrogen cyanide gas and are expensive because of the acid, electric current, and the heat necessary for neutralization. Batch treatment would be most desirable by any of these methods.

In the past, cyanide wastes were not thought to be amenable to biological treatment; however, recently some research has been conducted on the biological treatment of cyanide

wastes by a trickling filter. Pettet and Mills (14) have proven this treatment to be quite successful, and as a result there are a few experimental trickling filters treating cyanide wastes in England. The activated sludge process has never been used on a full scale plant for this purpose and there is little available information on the fundamentals of such a process. It will be the purpose of this study to investigate some of these fundamentals.

Review of Related Literature

Pettet, Mills, Painter, and Ware (11, 12, 13, 14, 15) of the Water Pollution Research Laboratory, Stevenage, England, have published most of the available literature concerning the aerobic biological treatment of cyanide wastes, with or without sewage. This biological treatment was accomplished by the use of a trickling filter, utilizing both heavy metal and simple salts of cyanide. At a concentration of 1.0 mg/l HCN in the presence of sewage, little effect upon the effluent B.O.D. was noticed; however, the nitrite concentration was unusually high and the nitrate concentration was appreciably reduced. Upon increasing the cyanide feed to 2.0 mg/l HCN the B.O.D. of the effluent increased and the nitrate concentration remained low. Nitrification returned to normal in an acclimated filter being fed 4.0 mg/l HCN, and increased above a sewage fed control filter in a filter acclimated to 150 mg/l HCN. At

this highest feed the cyanide residual in the effluent was less than 0.5 mg/l HCN and 50-100 percent of the nitrogen in the cyanide could be accounted for in the effluent as ammonia, nitrite, and nitrate. This nitrification indicated that the acclimated filter bacteria were using some of the cyanide as feed rather than being destroyed by its potential toxicity. Kittrell (5) suggested that the unaccounted for nitrogen may have been blown off as nitrogen gas, accumulated in the filter slimes, or converted to cyanates in the effluent.

Painter and Ware (11), working with Pettet, isolated a related gram-positive organism, provisionally classified among the Actinomycetaceae, capable of converting cyanide almost quantitatively to ammonia. The organism was aerobic, a strict autotroph, and lived ideally within a pH range of 7.5-9.0. Its growth was found to be very slow.

Trelawny, Schatz, Barth and Schatz (21) isolated organisms from soil samples which were able to metabolize both organic and inorganic cyanides. The isolated bacterium were aerobic gram-positive rods which required nitrates for growth. Their study also investigated the suitability of supplemental organic and nitrogenous compounds as sources of carbon and energy for growth on a basal medium containing 0.2 percent cyanoacetate. The results of these studies indicated that the organisms were not nitrifiers and could better assimilate cyanides when certain organic and nitrogenous compounds were also present.

Pettet and Mills (14) also conducted trickling filter

studies utilizing various cyanide compounds in the absence of sewage. After the sewage and cyanide acclimated filters were receiving 100 mg/l HCN, the sewage was gradually replaced with tap water. The cyanide concentration was then increased to 160 mg/l HCN, and the filters provided almost complete removal of the applied cyanide. Nitrates recovered in the effluent contained as high as 90 percent of the applied cyanide-nitrogen. These experiments showed that dilute solutions of wastes containing cyanides could be treated on a trickling filter to yield an effluent that, if not suitable for discharge into a watercourse, would at least be sufficiently purified for discharge into a sewer. No chemicals would be needed, since tap water or available domestic sewage could be used for the necessary bacterial nutrients. A few of these plants are in operation in England and have been found to be more economical than the present chemical treatment methods.

Activated sludge studies were also made at the British Water Pollution Research Laboratory (23). Their activated sludge unit contained 52,000 mg/l mixed liquor suspended solids and was fed, once each day, solutions of sodium cyanide containing cyanide in concentrations varying from 29 to 173 mg/l. These solutions provided cyanide feed ranging from 22 to 130 mg/l/day. Essentially 100 percent of the cyanide was removed at all feed rates, while total nitrogen recoveries varied from 85 to 110 percent. Of the

total nitrogen recovered, the percentage recovered as ammonia increased from 19 to 97 while the percentage recovered as nitrite plus nitrate decreased from 81 to 6 as the cyanide feed increased.

Activated sludge studies at the Robert A. Taft Sanitary Engineering Center on "The Biological Degradation of Nitriles" (4) indicated that suspended solids production by cyanide in the absence of sewage was not possible. However, Nolte and Brandt (10) found that normal replenishment of mixed liquor suspended solids could be achieved in an activated sludge system fed potassium cyanide in concentrations up to 330 mg/l KCN in the presence of calcium butyrate. They also found that all organisms except bacteria were destroyed above a concentration of 80 mg/l KCN. These results contradicted Wagner (22) who claimed the necessity of a bacterial-protozoan equilibrium for a satisfactory decomposition of organic matter. Nolte and Brandt (10) also concluded that the heavy metal ions were more inhibitory to bacterial metabolism than the cyanide ion.

Ludzack, Schaffer, Bloomhuff and Ettinger (8), using an acclimated biota from the Ohio River, were able to show that such surface water biota were able to metabolize a given nitrile in spite of wide variation in feed concentrations and feed intervals. The acclimated culture for a given nitrile converted 60 to 90 percent of the nitrile nitrogen in the feed to oxidized nitrogen. Later work by Ludzack,

Schaffer, and Bloomhuff (7) considered the treatability of organic cyanides by the activated sludge process and anaerobic digestion. Acclimation of the activated sludge to nitriles took a relatively short period of time and the sludge appeared quite resistant to shock loadings once acclimated. During normal operation of the system, 60 percent of the influent nitrogen as nitrile was oxidized. The organic cyanides proved to be toxic to the anaerobic digestion organisms, thus eliminating further consideration of this process for cyanide treatment.

Statement of the Problem

Previous research on the biological treatment of cyanogenic compounds has indicated these compounds to be amenable to this type treatment; however, little work has been done on the biological metabolism involved in the process. Therefore, it was the purpose of this research to study the metabolism of potassium cyanide in a laboratory scale activated sludge system. More specifically, the study included:

1. Experiments to determine whether cyanide was metabolized by the organisms or blown off as hydrogen cyanide gas in the process.
2. The use of two separate systems fed first at hour and then at half-hour intervals and receiving different total amounts of cyanide to determine an

equilibrium weight of suspended solids produced by each of these feeds.

3. The determination of a balance between the cyanide-nitrogen fed to the systems and the nitrogen recovered from the effluents of the systems.
4. The determination of a balance between the cyanide-carbon fed to the systems and the carbon dioxide produced by each system.
5. Determination of the effect on the system of the addition of inorganic and organic carbon to the cyanide feed.
6. The preliminary operation of a continuous flow unit.

Since previous work indicated that cyanide metabolism proceeded at an extremely low rate (11), a "no waste" activated sludge system was chosen for this study. This system would be comparable to the more recent total oxidation or ultimate oxidation systems where sludge removal is eliminated by oxidizing all excess activated sludge within the system.

This study was not expected to provide specific data for the design of a waste treatment plant, since cyanide wastes contain heavy metal and organic complexes as well as the simple salts of the cyanide ion. However, it would be of value to future investigators who might be searching for design data, inhibiting factors, and practicability of an activated sludge system to treat complex cyanide wastes.

II. APPARATUS AND EXPERIMENTAL PROCEDURE

Apparatus - Batch

The apparatus used to maintain the batch activated sludge cultures in this research is shown in Figures 1 and 2. It consisted of two, stoppered, 3-liter cylinders held in place in a constant temperature water bath at 24°C. Attached to each cylinder through the stopper was a cyanide feed hose, an air supply hose containing a carborundum air diffuser, and an exhaust air hose. The exhaust air hose was attached to a gas washer containing sodium hydroxide at pH 11+ to collect any hydrogen cyanide gas blown from the system.

The potassium cyanide feed apparatus is shown in detail in Figure 2. When the arm of the clock motor moved to position "A", valve "a" (rubber hose from the cyanide reservoir connected to a stationary glass tee) opened, allowing a known quantity of potassium cyanide to flow into the glass tee and hose. In this position valve "b" was closed. When the arm on the clock motor moved to position "B", valve "b" opened, allowing the known quantity of potassium cyanide to flow into the cylinder. A capillary tube was used on the end of the calibrated hose and glass tee so that varying liquid levels in the cyanide reservoir did not appreciably affect the hourly volume of cyanide fed.

The ten-liter culture apparatus shown in Figure 1 was

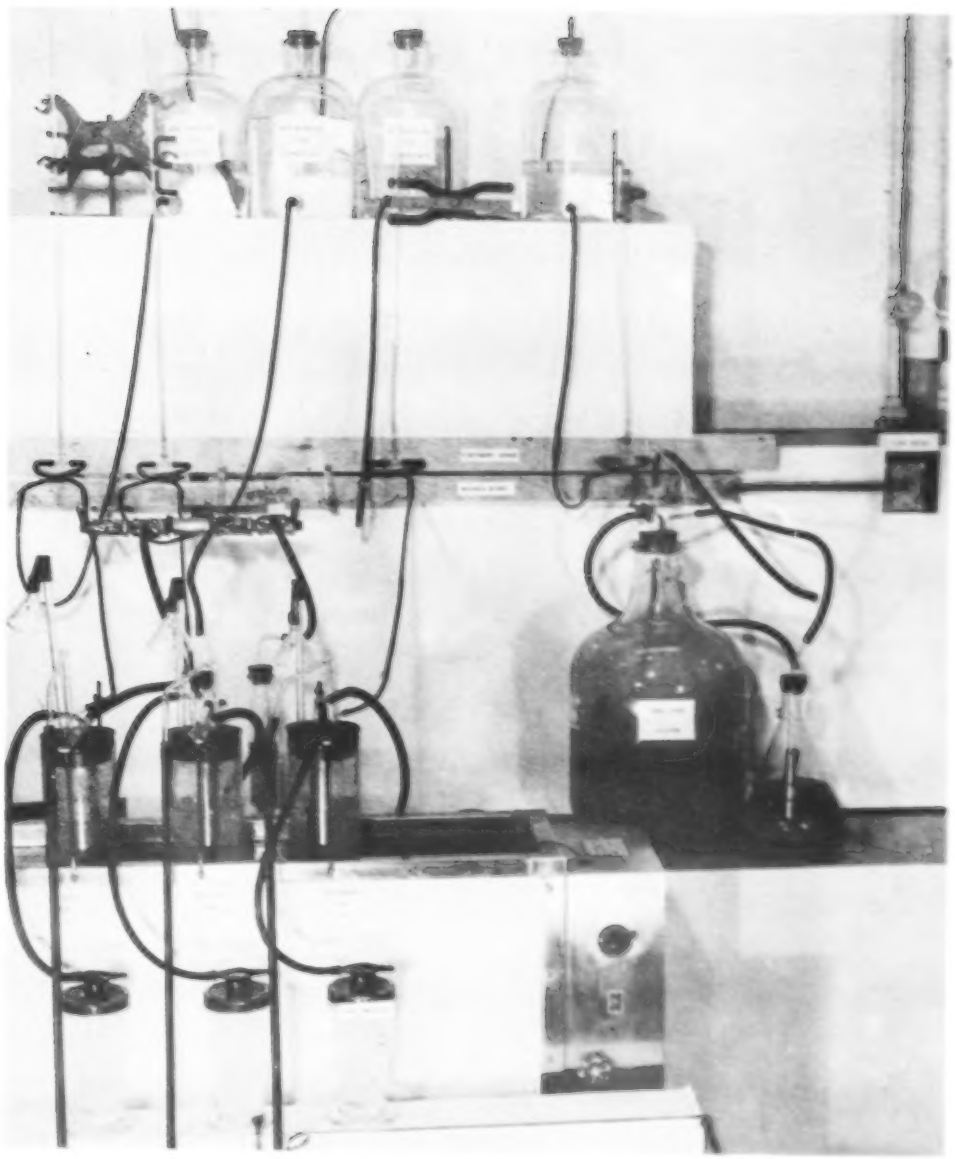

FIGURE 1 - EXPERIMENTAL APPARATUS

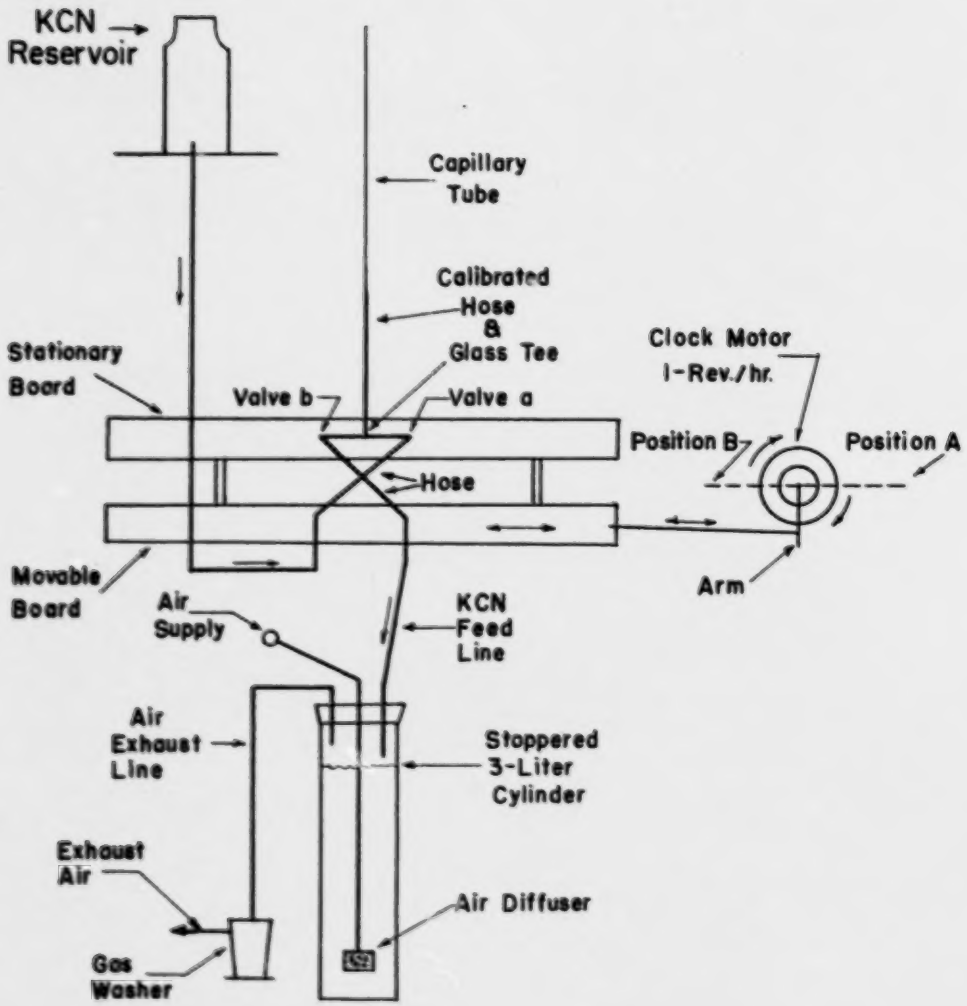

FIGURE 2 EXPERIMENTAL APPARATUS

identical to that of the cylinders except for the volume of the culture container.

Apparatus - Continuous Flow

The apparatus used for the continuous flow studies in this research is shown in Figures 3 and 4. It consisted of a single 100 liter tank containing an 84 liter aeration chamber and a 16 liter settling chamber. A constant head device maintained a flow of 278.5 milliliters per minute of tap water through both chambers. This flow resulted in an aeration time of 3.7 hours and a surface overflow rate in the settling chamber of 108 gallons per day per square foot of chamber surface area. The temperature of the influent tap water was controlled at approximately 20°C. by passing the water through a coil of Tygon tubing immersed in a hot water bath. A Fisher and Porter flowrator measured the 20,000 cubic centimeters per minute of air that was blown through four carborundum diffusers fastened in the bottom of the aeration chamber. Oxygen transfer tests with sodium sulfite indicated that this air flow transferred 200 mg per minute of oxygen to the aerated liquor (See Appendix B). Potassium cyanide solution was fed from a cyanide reservoir to the aeration chamber at a rate of 4.2 milliliters per minute by a Precision pump. Both the tap water and cyanide feed rates could be varied considerably if desired.

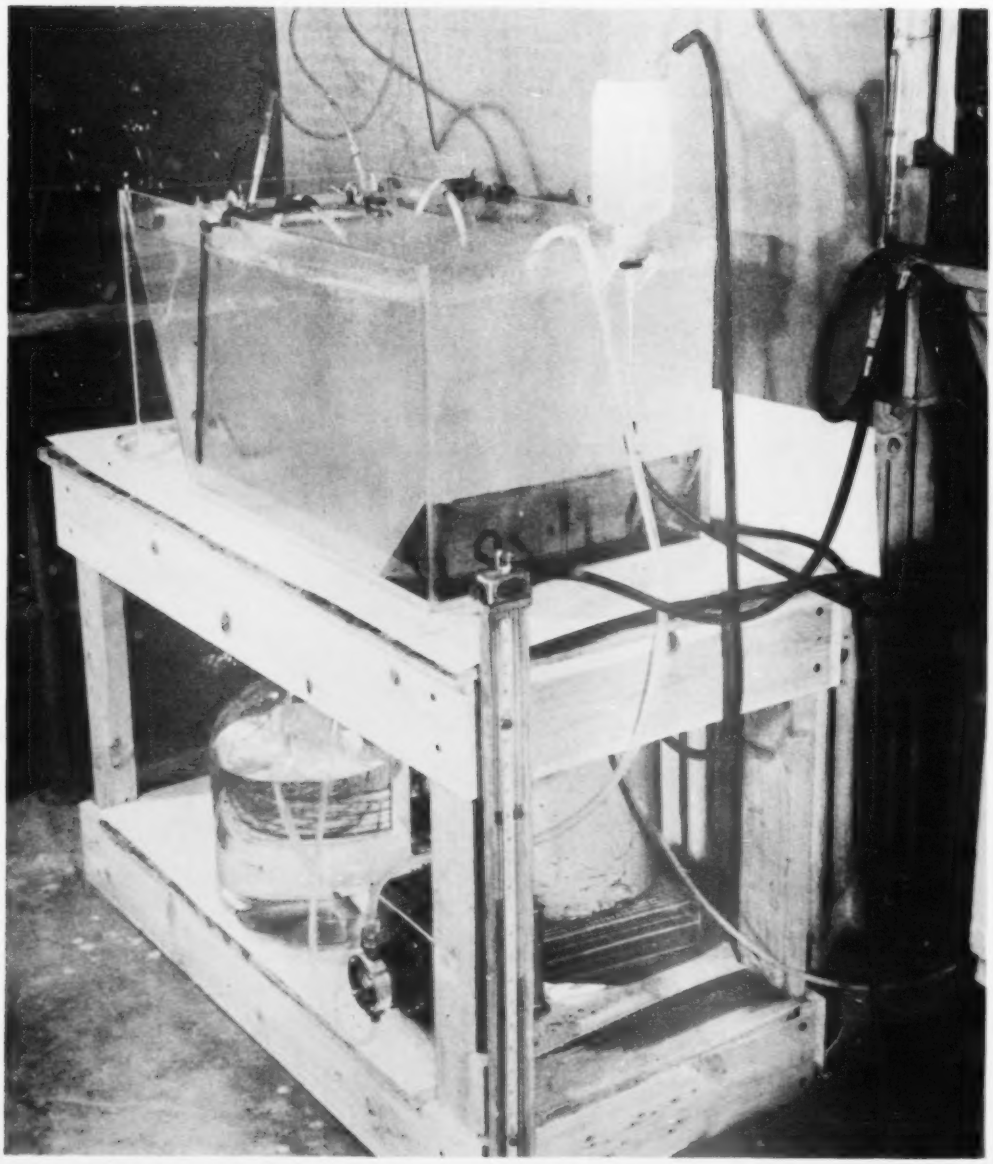

FIGURE 3 - CONTINUOUS FLOW PILOT PLANT

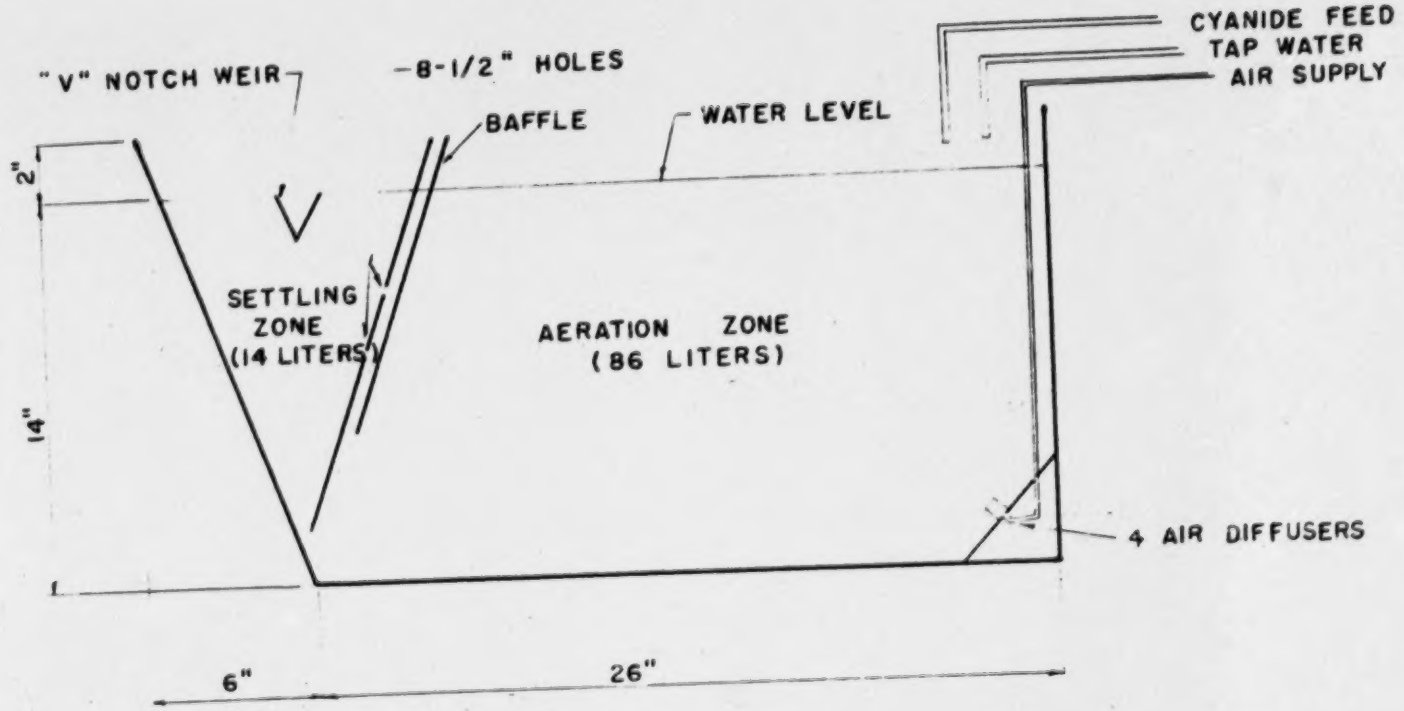

FIGURE 4 - CONTINUOUS FLOW PILOT PLANT

Analytical Tests Employed

The following tests were employed for the quantitative analyses used throughout this research:

A. Suspended Solids

Procedure:

1. Dry a 5.0 cm aluminum dish and No. 1 (4.25 cm) or fiberglass filter paper in an oven at 103°C. for two hours.
2. Cool in a dessicator and weigh.
3. Place the filter paper in a millipore filter holder, place the holder in a vacuum flask and apply 20 in. vacuum to the flask.
4. Immediately add the suspended solids sample to the filter holder.
5. Remove the filter paper from the holder, place the paper in the aluminum dish and dry in a 103°C. oven for two hours.
6. Place the dry dish and paper in a dessicator to cool and then weigh.
7. Determine the weight of the sampled solids by subtracting the two weighings.
8. The suspended solids concentration, in mg/l, is equal to:

$$\frac{(\text{wt. of sampled solids, mg}) \times 1000}{\text{Sample size, ml}}$$

B. Volatile Solids

Performed on the suspended solids residue as specified in Standard Methods for the Examination of Water, Sewage, and Industrial Wastes (1).

C. Cyanide

Standard titration method for cyanides as specified in Standard Methods for the Examination of Water, Sewage, and Industrial Wastes (1).

D. pH

pH was determined electrometrically with a Beckman pH meter.

E. Ammonia

Standard nesslerization test for ammonia using the Calgon modification (2).

F. Nitrites

Standard test for nitrites as specified in Hellige Technical Information Bulletin No. 611-20, Hellige, Inc., Long Island City, New York.

G. Nitrates

Standard phenoldisulfonic acid method as specified in Standard Methods for the Examination of Water, Sewage, and Industrial Wastes (1).

H. Organic Nitrogen

Standard test for organic nitrogen as specified in Standard Methods for the Examination of Water, Sewage, and Industrial Wastes (1).

I. Carbon Dioxide

Collection in a Fisher-Milligan gas washer

containing a solution of potassium hydroxide at pH 11+. Potassium carbonate was precipitated by 0.2N barium chloride from a 50 ml portion taken from the gas washer. The barium carbonate precipitate was filtered through a fiberglass filter, dried at 700°C. in a muffle furnace, and weighed.

J. Chemical Oxygen Demand (COD)

Standard test as prepared by Professor Robert S. Ingols for the Eleventh Edition of Standard Methods for the Examination of Water, Sewage and Industrial Wastes (1).

Experimental Procedure - Batch Operation

General:

In order to acclimate activated sludge to potassium cyanide, ten liters of normal activated sludge were first developed by aerating 100 ml of primary sewage sludge from the State College Sewage Treatment Plant in ten liters of tap water. The culture was settled for one hour daily, five liters of clear supernatant decanted and replaced with tap water and 100 ml of primary sludge. No solids were withdrawn from the system at any time.

After a culture of activated sludge was developed (mixed liquor suspended solids concentration of 2000 mg/l), 10 mg CN^- was added daily in hourly increments. The cyanide residual was determined daily on the settled effluent and when no cyanide residual was found, cyanide was increased to

20 mg CN^- per day. About seven days were required for the culture to completely metabolize the 10 mg CN^- feed and produce no residual cyanide. This procedure was followed until the daily cyanide feed was 400 mg CN^-. At this point the 100 ml sludge feed and the tap water were gradually replaced with dilution water containing 22.5 mg/l $MgSO_4 \cdot 7H_2O$, 27.5 mg/l $CaCl_2$, 0.25 mg/l $FeCl_3 \cdot 6H_2O$, 885 mg/l $Na_2HPO_4 \cdot 7H_2O$, and sufficient NaH_2PO_4 to bring the pH to 7.0. Thus, by this replacement, the cyanide became the only source of carbon and nitrogen for the culture. The cyanide reservoir was also buffered to pH 8.9 as experience proved this necessary to keep the system operating in the pH range 7.5-8.5.

Run No. 1:

When the ten-liter culture was metabolizing 400 mg CN^- per day and producing no cyanide in the effluent, one liter of the mixed liquor was transferred to each of two three-liter cylinders and diluted to three liters with the buffered dilution water. For the duration of the run, one cylinder was fed 60 mg CN^- per day and the other 120 mg CN^- per day through the hourly feed system. The ten-liter culture was reacclimated to 400 mg CN^- per day and kept in reserve.

The following was a schedule of the normal daily operating procedure:

1. Cut off the cyanide feed and air supply and allow each cylinder to settle for one hour. From the

166th day to the end of the run the cylinder walls were washed before settling.

2. Decant one liter of the settled liquor (effluent) from each cylinder.
3. Filter the effluent through clean sand, wash the sand with dilution water and return the dilution water and washed solids to the respective cylinder.
4. Fill each cylinder to the three liter mark with dilution water and resume aeration and the cyanide feed.
5. Determine the cyanide residual and the pH of the effluent.
6. Determine the amount of cyanide in the gas washers.

Approximately once a week samples of mixed liquor were withdrawn for the determination of suspended solids and periodically for organic nitrogen.

Daily measurements of ammonia, nitrite, and nitrate nitrogen were made on the effluent prior to filtration during the time a nitrogen balance was being made.

When a carbon balance was being run the air supply to each cylinder was passed through a soda-lime column to remove all incoming carbon dioxide (Figure 5). The exhaust air was then passed through a Fisher-Milligan gas washer containing potassium hydroxide which collected the carbon dioxide given off during the metabolism. Barium chloride and moisture traps were placed between the soda-lime column and the culture

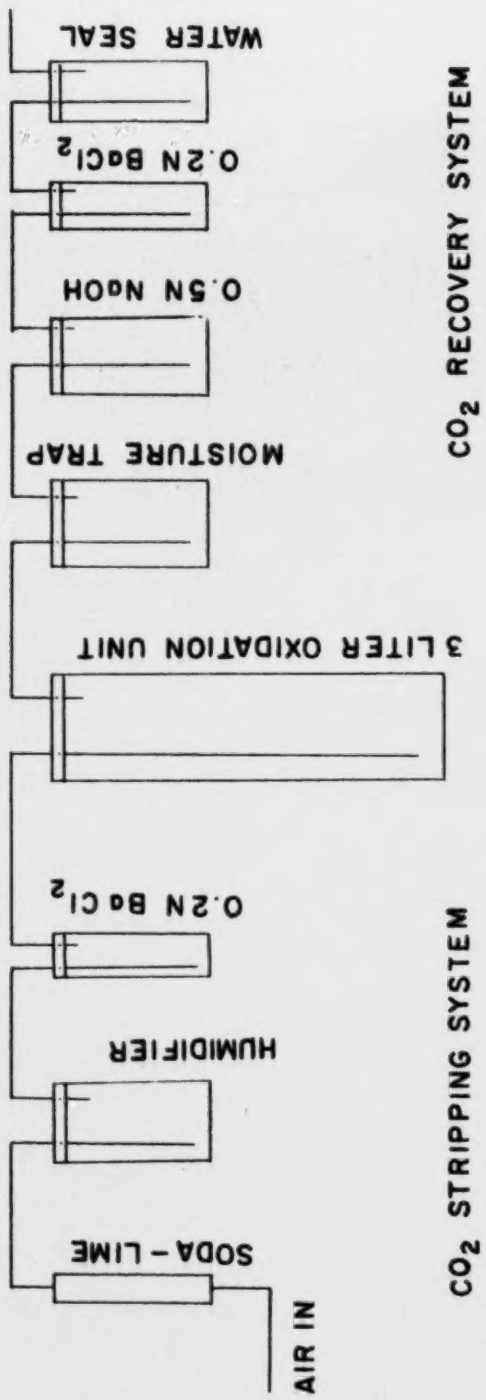

FIGURE 5 - CARBON RECOVERY APPARATUS

tube and after the gas washer to indicate whether any carbon dioxide passed either device. A water seal was placed at the end of the system to eliminate the entrance of atmospheric carbon dioxide.

Run No. 2:

Run No. 2 was similar to Run No. 1 except that cyanide was fed to each of the two cylinders at half-hour intervals, the cylinder walls were washed daily during the entire run, and volatile solids were also determined on the mixed liquor. The feed rate was such that the two cylinders received 120 mg CN^- per day and 180 mg CN^- per day respectively.

Run No. 3:

In Run No. 3 each of two, three-liter cylinders was fed 120 mg CN^- per day at half hour intervals. In one cylinder the cyanide served as the only source of carbon and nitrogen for the organisms. In the other cylinder the feed carbon was supplemented with sodium acetate sufficient to give a COD/N of approximately 29/1 during the greater part of the run.

Periodically samples of mixed liquor were withdrawn for the determination of suspended and volatile solids and organic nitrogen. Daily samples of the effluent were taken for the determination of COD, soluble organic nitrogen, ammonia, nitrites and nitrates.

Experimental Procedure - Continuous Operation

To start the continuous feed pilot plant a twelve-liter seed culture of microorganisms previously acclimated to cyanide in the ten-liter jug described in the batch-operation procedure was acclimated to a feed of tap water and 240 mg CN^- per day. The acclimated culture was transferred to the pilot plant which was operated as previously described with the cyanide feed pump set to feed 240 mg CN^- per day. This cyanide feed rate produced an equivalent cyanide concentration in the influent tap water of 0.44 mg/l.

The pilot plant was operated for 31 days during which time the cyanide concentration in the influent tap water was increased by increments to 7.35 mg/l as shown in Table 17. Periodic samplings of the effluent were taken for the determination of cyanide concentration.

III. RESULTS

Batch Operation

Residual Cyanide

Table 1 shows residual cyanide data typical of two types of operation that existed during the three runs of the experiment. Days 31 through 35 on Cylinder No. 2 and 69 through 72 on Cylinder No. 3 are typical of the normal operation that existed during the greater part of the experiment when no residual cyanide was found in the supernatant and less than 0.3 mg CN^- in the gas washers.

Several times during the experiment mechanical difficulties such as large variations in the cyanide feed rate or loss of air for several hours occurred. Days 36 through 54 in Cylinder No. 2 and 73 through 81 in Cylinder No. 3 are typical operating results during such a period. At this time residual cyanide was recovered in both the supernatant and gas washers. In order to return to normal operation following a mechanical difficulty, it was necessary immediately to reduce the cyanide feed rate to one that could be metabolized by the system (usually one-half the normal rate) and gradually increase it to regain the normal rate.

TABLE 1

RESIDUAL CYANIDE DATA

Day	Cylinder No. 2			Day	Cylinder No. 3		
	CN^- fed mg	Residual CN^- mg	CN^- gas washer mg		CN^- fed mg	Residual CN^- mg	CN^- gas washer mg
31	120	0.0	0.2	69	60	0.0	0.1
32	120	0.0	0.2	70	60	0.0	0.2
33	120	0.0	0.2	71	60	0.9	0.1
34	120	0.0	0.3	72	60	0.0	0.0
35	120	0.1	0.3	73	0	0.0	0.0
36	120	69.0	9.1	74	60	15.2	2.4
37	60	0.5	0.3	75	60	20.6	3.6
38	60	27.8	4.9	76	60	24.8	3.6
50	105	0.0	0.1	77	60	27.8	4.0
51	105	0.0	0.1	78	36	16.5	3.8
52	105	0.0	0.1	79	36	0.0	--
53	105	0.0	0.1	80	36	0.8	0.1
54	105	0.0	0.1	81	36	0.0	--

To insure that all hydrogen cyanide gas blown from the systems would be collected in a single gas washer, the effluent air from a three-liter solution containing 60 mg CN^- was connected to two gas washers containing sodium hydroxide (pH 11+). This solution was aerated for a period of 11 days and tests were conducted daily to determine the amount of cyanide blown from the system and that remaining in solution. It will be noted in Table 2 that an extremely small amount of cyanide was sparged into a second gas washer from a low concentration of cyanide in solution. Since a concentration of 5.0 mg CN^- was the maximum normal concentration present in the cylinders at any one time, it was

assumed that essentially all of the cyanide blown from the cylinders was collected in one gas washer.

TABLE 2

TEST CYANIDE SOLUTION DATA

Day	CN^- concentration in cylinder mg	Residual CN^- in cylinder percent	Residual CN^- 1st Washer percent	Residual CN^- 2nd Washer percent
1	46.9	78.2	19.5	2.3
2	38.0	81.0	17.7	1.3
3	28.5	75.0	22.4	2.6
4	23.3	81.6	16.1	2.3
5	22.0	94.5	3.9	1.6
6	20.5	93.2	4.5	2.3
7	19.0	92.6	4.9	2.5
8	18.0	94.6	4.2	1.2
9	17.1	95.0	4.5	0.5
10	16.3	95.5	4.1	0.4
11	15.8	97.0	2.5	0.5

Mixed Liquor Suspended Solids

Table 3 and Figure 6 show the cyanide feed and suspended solids concentrations throughout the 185 days of operation of Run 1.

During the first 40 days of operation, mixed liquor suspended solids were determined daily and the liter of supernatant liquor decanted daily from each cylinder was discarded. Using this procedure, the suspended solids were quite erratic and showed a steady decline to concentrations of 130 mg/l and 100 mg/l in Cylinders 2 and 3 respectively.

There was no indication that an equilibrium would be obtained.

TABLE 3

CYANIDE FEED - SUSPENDED SOLIDS DATA

Run 1

Day	Cylinder No. 2		Cylinder No. 3	
	CN^- Feed mg/day	Suspended Solids mg/l	CN^- Feed mg/day	Suspended Solids mg/l
1	60	710	60	284
11	120	444	60	201
33	120	132	60	30
42	60 (1)	102	60	107
45	75	109	60	74
49	105	139	60	136
52	105	138	60	131
57	120	142	60	136
68	120	200	60	166
71	120	196	60	138
81	120	212	36 (2)	113
96	102 (3)	226	60	112
123	120	121	60	63
131	120	234 (4)	60	252 (4)
134	120	38	60	70
135	120	60	60	108
152	109	288	58	152
158	120	134	60	148
160	120	112	60	60
166	120	146	60	166
169	120	166	60	192
171	120	160	60	228
174	120	170	60	310
177	120	154	60	230
180	120	140	60	220
182	120	126	60	192
185	120	130	60	200

1. Mechanical failure required the reduction of the CN^- feed to 60 mg/day on the 37th day. It returned to 120 mg/day on the 56th day.
2. Mechanical failure required the reduction of the CN^- feed to 36 mg/day on the 78th day. It returned to 60 mg/day on the 90th day.
3. Mechanical failure required the reduction of the CN^- feed to 90 mg/day on the 85th day. It returned to 120 mg/day on the 115th day.
4. Daily sampling for Org.-N and/or ss. 131st to 140th days.

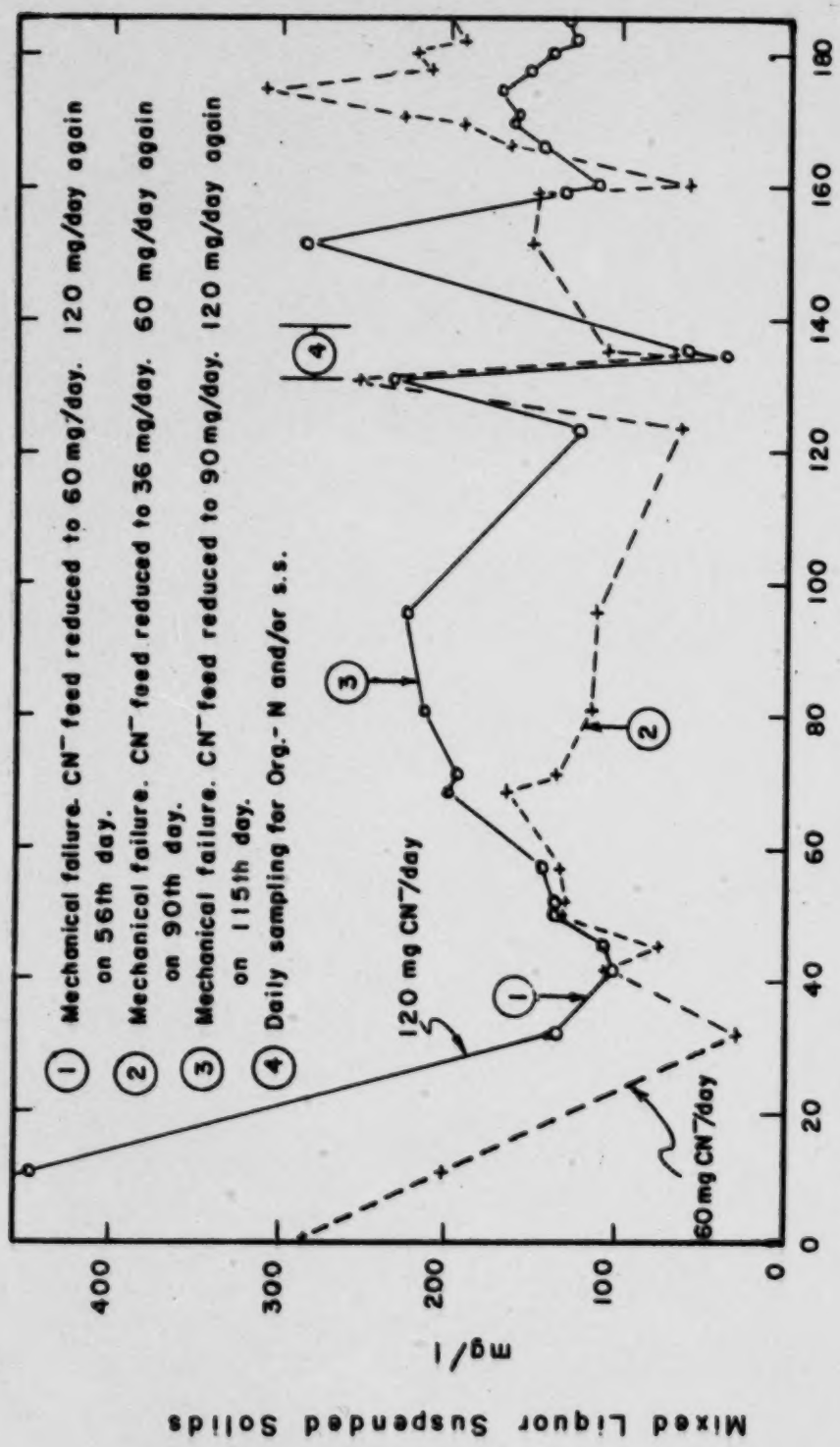

FIGURE 6 - MIXED LIQUOR SUSPENDED SOLIDS

From the 40th to 115th day of operation, less frequent suspended solids tests were taken and the daily effluent was filtered through sand with the solids returned to the systems. During this period the suspended solids concentrations increased to approximately 220 mg/l and 110 mg/l in Cylinders 2 and 3 respectively. However, due to mechanical difficulties and varying cyanide feed concentrations, it was impossible to maintain any consistent suspended solids concentrations during this period.

The cyanide feed concentration remained normal from the 115th day to the end of the experiment; however, the results of the suspended solids determinations were somewhat erratic until the 166th day. These erratic results undoubtedly were caused by daily sampling for organic nitrogen and/or suspended solids from the 131st to 138th days and an appreciable accumulation of solids on the cylinder walls. This accumulation of solids had been noticed prior to the 115th day and could have been partially responsible for the erratic data prior to that time. From the 166th day to the end of the experiment the cylinder walls were washed daily and more consistent suspended solids results were obtained.

The cyanide feed and suspended solids data for Run 2 are shown in Table 4 and Figure 7.

The cyanide feed remained fairly constant during the run with the exception of the period from the 44th to the 66th day in Cylinder No. 2. On the 44th day a mechanical

TABLE 4

CYANIDE FEED - SUSPENDED SOLIDS DATA

Run 2

Day	Cylinder No. 2 (2)			Cylinder No. 3 (2)		
	CN^- Feed mg/day	M.L.S.S. mg/l	Volatile Solids %	CN^- Feed mg/day	M.L.S.S. mg/l	Volatile Solids %
5	--	--	--	120.0	190	--
12	--	--	--	120.0	148	--
30	175.8	350	--	120.0	252	--
34	175.8	312	--	120.0	412	--
39	175.8	620	--	120.0	266	--
49	126.6 (1)	636	85	122.4	304	91
56	145.8	394	80	122.4	170	84
63	154.2	386	87	117.6	294	92
67	176.1	578	76	117.6	246	91
70	176.1	440	96	117.6	228	89
74	176.1	422	--	124.8	250	--
77	176.1	586	72	124.8	274	90
81	176.1	444	87	124.8	220	92
		Average	83		Average	90

(1) Mechanical failure required the reduction of the CN^- feed to 126.6 mg/day on the 44th day. It returned to 176.1 mg/day on the 66th day.

(2) Except on the 44th to 46th days in Cylinder 2 no residual cyanide was detected in either cylinder during the run.

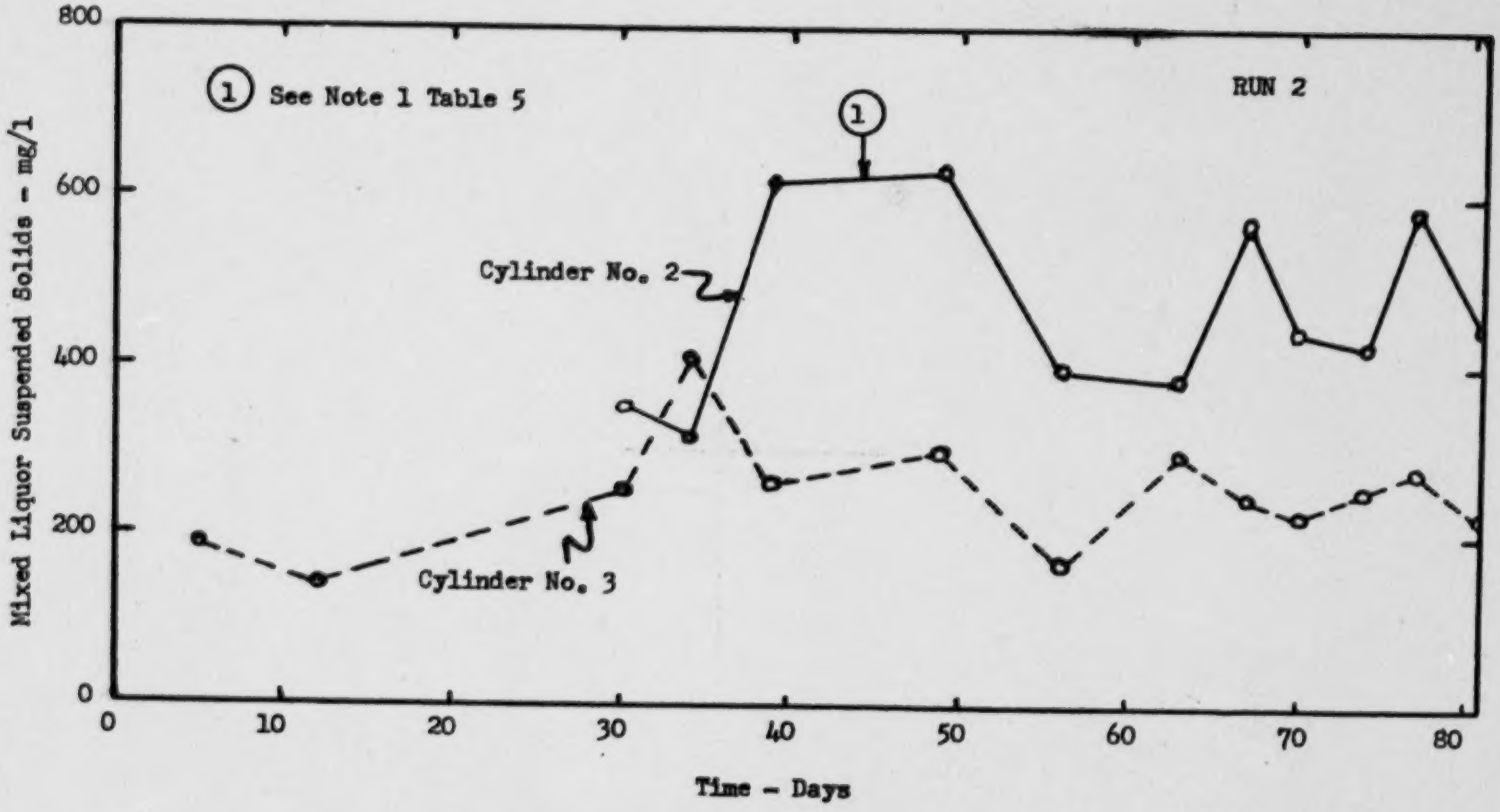

FIGURE 7 - MIXED LIQUOR SUSPENDED SOLIDS

failure in this cylinder upset its metabolism creating a cyanide residual and forcing a reduction in the cyanide feed. The cyanide residual disappeared on the 46th day and the cyanide feed was increased gradually until it returned to normal on the 66th day.

For Run 3 the cyanide feed and suspended solids data are shown in Table 5 and Figure 8. The cyanide feed remained at approximately 120 mg/day in both cylinders during the entire run and no mechanical failures occurred. During the first four days of the run cyanide served as the only source of carbon and nitrogen for the organisms in both cylinders and the mixed liquor suspended solids in both cylinders averaged about 240 mg/l.

On the fifth day sufficient sodium acetate was added to the reservoir for Cylinder 2 to feed 4270 mg COD/day to the cylinder and make the C/N in the feed 25/1. At this COD feed rate the pH in the cylinder increased to 9.5-10.0 and was difficult to lower; however, no cyanide residual was detected at any time and a reasonable percentage removal of COD was realized. Because of the high pH, the sodium acetate concentration in the reservoir was reduced on the 10th day to feed 1780 mg COD/day to the cylinder and make the COD/N in the feed approximately 29/1.

From the 13th day to the end of the run the culture in the cylinder removed about 95 percent of the COD added per day. The mixed liquor suspended solids remained about 94 percent volatile and their concentration increased steadily

TABLE 5

FEED, SUSPENDED SOLIDS AND EFFLUENT DATA

Run 3

Day	Cylinder No. 2 (1) COD - mg/day Feed (2)	Effluent	Percent Removed	M.L. Suspended Solids Total mg/l	Volatile mg/l	% of ss	Cylinder No. 3 (1) M.L. Suspended Solids Total mg/l	Volatile mg/l	% of ss
1	--	--	--	210	--	--	160	--	--
2	--	--	--	270	--	--	232	--	--
5	--	--	--	214	--	--	240	--	--
9	4270	1552	64	--	--	--	--	--	--
10	4270	835	80	506	--	--	230	--	--
13	1780	--	--	596	--	--	252	--	--
16	1780	83	95	686	--	--	290	--	--
17	1780	106	94	816	780	96	--	--	--
18	1780	104	94	880	--	--	--	--	--
19	1780	82	95	920	--	--	--	--	--
20	1780	52	97	1040	980	94	258	240	93
21	--	--	--	1172	1024	87	--	--	--
22	2360	163	93	1240	1162	94	--	--	--
23	2360	53	97	1216	1166	96	--	--	--
24	2360	75	97	1262	1120	89	268	236	88

(1) CN^- feed during the entire run was approximately 120 mg/day. No residual CN^- was found at any time.

(2) COD resulted from sodium acetate and potassium cyanide in feed solution.

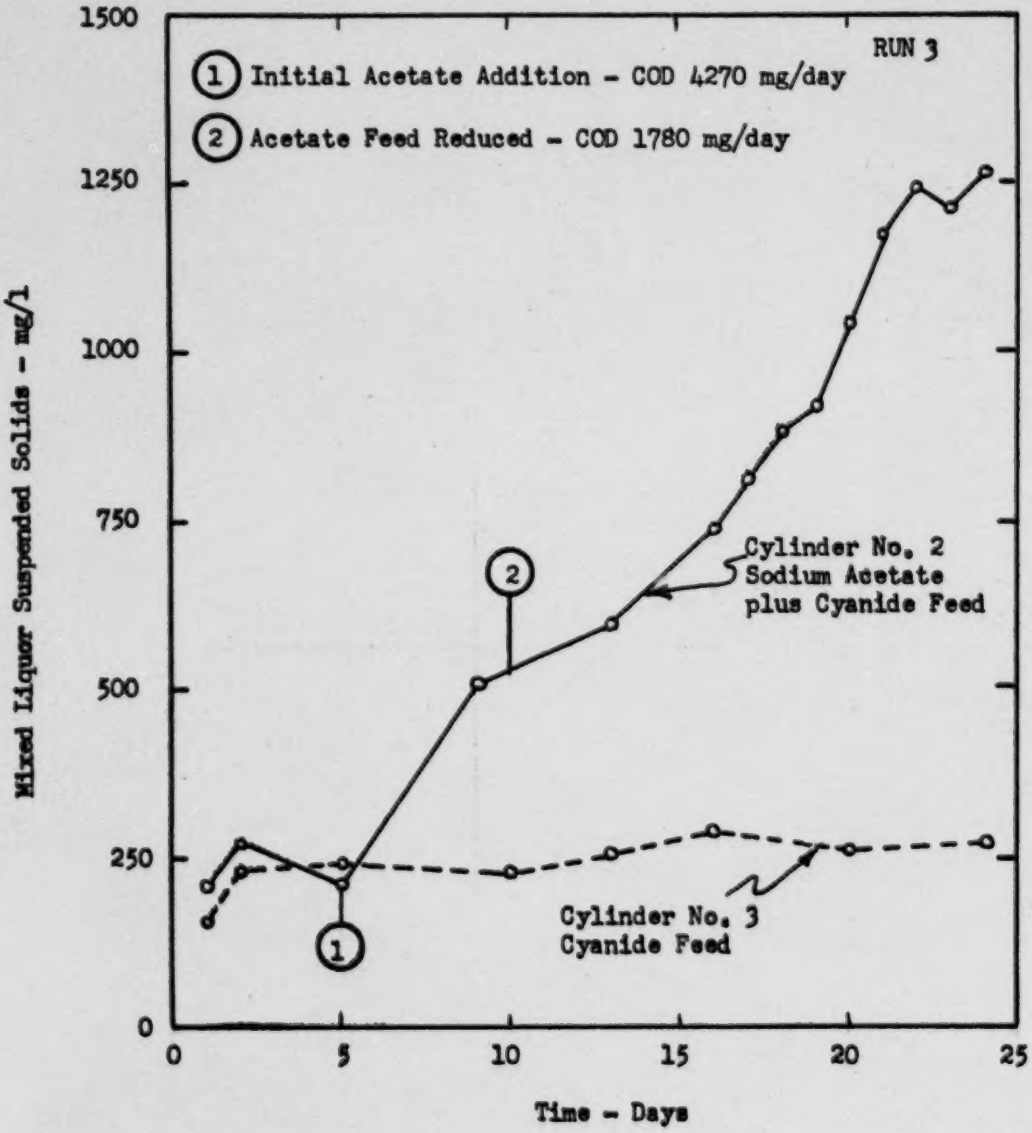

FIGURE 8 - MIXED LIQUOR SUSPENDED SOLIDS

to about 1250 mg/l. On the 22nd day their concentration appeared to reach a slowly rising plateau; however, time did not permit the experiment to be continued long enough to completely define this plateau.

During the entire run the suspended solids concentration in the control cylinder (No. 3) remained at about 250 mg/l with about 90 percent of their weight being volatile.

Nitrogen

At intervals between the 115th and 185th day of Run 1 nitrogen recovery tests were conducted on the effluent of both cylinders to determine the weights of ammonia, nitrite, and nitrate produced through the cyanide metabolism. Tables 6 and 7 list the weights and percentages of these products found and also the total percentage nitrogen recovered for each of the cultures during two different periods.

For the first period (Table 6) nitrogen recovery in the effluent of Cylinder No. 2 averaged 14.2 percent ammonia, 70.0 percent nitrite, 15.8 percent nitrate, for a total nitrogen recovery of 77.7 percent. A 90.0 percent nitrogen recovery was obtained in the effluent of Cylinder No. 3 with 14.7 percent as ammonia, 50.0 percent as nitrite, and 35.3 percent as nitrate.

During the second nitrogen recovery period (Table 7) 75.0 percent of the nitrogen added was recovered in the

TABLE 6

NITROGEN RECOVERY DATA
(1st Period)

Run 1

Day	Cylinder No. 2					Cylinder No. 3				
	NH_3-N mg/l	NO_2-N mg/l	NO_3-N mg/l	N-gain* mg	CN-N fed** mg	NH_3-N mg/l	NO_2-N mg/l	NO_3-N mg/l	N-gain* mg	CN-N fed** mg
131	8.1	27.0	6.3	--	--	4.3	12.8	8.4	--	--
132	10.1	28.5	6.4	57.8	63.5	3.8	14.3	8.9	33.3	33.6
133	7.8	31.5	5.8	48.7	61.0	3.4	15.0	8.7	29.0	32.3
134	6.1	33.0	6.9	51.2	61.0	4.5	15.3	8.4	32.6	32.3
135	5.0	30.5	5.9	37.3	63.5	3.9	13.5	9.2	26.7	33.6
136	5.2	25.5	6.7	34.2	61.0	4.3	12.0	9.1	26.5	32.3
137	5.6	26.5	7.0	39.9	59.0	4.1	9.8	10.7	22.9	31.4
138	4.5	33.5	7.2	64.8	59.0	3.3	12.8	9.6	31.0	31.4
139	4.6	32.0	7.4	41.4	59.0	3.7	13.0	10.1	29.2	31.4
140	4.1	33.5	8.0	49.0	59.0	3.8	13.8	10.0	29.3	31.4
				424.3	546.0				260.5	289.7
Average	6.1	30.1	6.8			3.9	13.2	9.3		
% of Total	14.2	70.0	15.8			14.7	50.0	35.3		

% of recovery = $\frac{424.3}{546.0}$ x 100 = 77.7%

% of recovery = $\frac{260.5}{289.7}$ x 100 = 90.0%

* See Appendix for sample calculation.
** Feed at hourly intervals.

TABLE 7

NITROGEN RECOVERY DATA
(2nd period)

Run 1

Day	Cylinder No. 2					Cylinder No. 3				
	NH_3-N mg/l	NO_2-N mg/l	NO_3-N mg/l	N-gain* mg	CN-N fed** mg	NH_3-N mg/l	NO_2-N mg/l	NO_3-N mg/l	N-gain* mg	CN-N fed** mg
154	3.0	31.5	10.6	--	--					
155	3.1	32.0	12.0	51.2	64.6	1.1	7.5	17.7	--	--
156	2.6	34.5	11.3	51.0	64.6	1.3	8.3	19.2	39.1	32.5
157	2.5	37.5	10.7	50.0	64.6	0.0	7.0	22.6	32.0	32.5
158	2.5	36.5	11.5	50.0	67.5	0.3	7.0	21.0	24.9	32.5
159	3.0	33.0	11.8	48.0	64.6	0.0	3.0	22.5	19.5	33.8
160	3.5	32.0	10.0	41.0	64.6	1.5	5.3	20.8	32.0	32.5
161	3.4	40.0	11.3	74.5	64.6	0.0	4.1	19.3	14.5	21.6
162	3.7	33.0	9.7	25.0	64.6	0.2	5.0	18.2	23.4	33.8
163	0.0	32.0	12.8	44.2	32.8	0.5	4.1	19.0	23.9	33.8
164	4.7	24.0	12.0	32.0	62.0	0.8	6.0	20.8	36.0	34.7
165	6.0	29.0	10.3	54.9	67.5	1.0	5.5	21.3	28.5	32.0
166	6.8	30.5	10.3	53.2	64.6	1.2	5.7	22.2	31.4	34.7
167	8.4	30.0	12.0	64.5	67.5	1.0	4.0	25.0	31.9	33.4
168	8.0	24.0	10.0	24.0	64.6	1.3	6.6	21.0	33.7	34.7
169	6.8	28.5	13.7	64.0	64.6	1.6	5.8	22.4	26.6	33.4
				707.5	943.3	1.7	5.8	23.0	33.8	33.4
									431.0	499.3
Average	4.3	31.8	11.3							
% of Total	9.1	67.0	23.9			0.8	5.7	21.0		
						2.9	20.7	76.4		

% of recovery = $\frac{707.5}{943.3}$ x 100

= 75.0%

% of recovery = $\frac{431.0}{499.3}$ x 100

= 86.3%

* See Appendix for sample calculation
** Feed at hourly intervals

TABLE 8

SUSPENDED SOLIDS-ORGANIC NITROGEN DATA

Run 1

Day	Cylinder No. 2			Cylinder No. 3		
	Suspended solids mg/l	Organic-N mg/l	N/Suspended solids percent	Suspended solids mg/l	Organic-N mg/l	N/Suspended solids percent
134	--	--	--			
135	60	6.0	10.0	70.0	4.5	6.5
166	146	13.2	9.0	108	6.9	6.4
169	166	13.0	8.1	166	11.4	6.9
171	160	12.9	8.1	192	13.5	6.9
174	170	13.8	8.1	228	15.9	7.0
177	154	13.2	8.6	310	16.6	5.4
				230	14.4	6.3
		Average	8.6		Average	6.5

effluent of Cylinder No. 2, with 9.1 percent as ammonia, 67.0 percent as nitrite, and 23.9 percent as nitrate. During this same period 86.3 percent of the nitrogen fed to Cylinder No. 3 was recovered, with the effluent averaging 2.9 percent ammonia, 20.7 percent nitrite, and 76.4 percent nitrate.

At no time during the nitrogen recovery tests was an appreciable amount of ammonia recovered in the gas washers.

Organic nitrogen tests were conducted at various times throughout the run. Table 8 lists the organic nitrogen found, suspended solids at the time of sampling, and the percentage nitrogen by weight in the suspended solids. The percentage organic nitrogen averaged 8.6 percent and 6.5 percent in Cylinders 2 and 3, respectively.

Similar nitrogen recovery and organic nitrogen tests were made during Run 2. Data from these tests are presented in Tables 9, 10, and 11 and Figures 9 and 10.

During this run the nitrogen recovered in the effluent of the cylinders as ammonia, nitrite and nitrate averaged 94 percent and 100 percent of the cyanide nitrogen added for Cylinders 2 and 3 respectively. The distribution of the recovered nitrogen among these three forms was not constant during the run but varied in both cylinders as shown in Figures 9 and 10. The mechanical failure which upset Cylinder 2 on the 44th day also caused an increase in the percentage of effluent nitrogen recovered as nitrite

TABLE 9

NITROGEN RECOVERY DATA

Run 2, Cylinder 2

Day	NH_3-N mg/l	NO_2-N mg/l	NO_3-N mg/l	Inorganic-N mg/l	CN-N fed mg/l	N-Recovery mg/l
40	3.8	3.0	85.0	91.8	90.9	100
41	2.3	5.6	88.5	96.4	90.9	103
42	3.0	4.9	90.0	97.9	90.9	104
48	3.5	30.0	17.2	50.7	65.2	78
49	4.1	32.8	15.5	52.4	65.2	80
53	4.4	33.0	19.5	56.9	65.2	88
54	4.1	27.0	17.3	49.4	65.2	76
55	4.7	22.5	21.8	49.5	65.2	76
56	4.1	31.0	44.2	79.3	65.2	122
59	4.2	8.6	58.0	70.8	75.2	94
60	4.2	2.4	62.0	68.8	75.2	92
61	4.0	2.1	70.0	76.1	79.5	96
62	5.2	2.2	75.0	82.4	79.5	103
63	4.4	1.6	70.0	76.0	79.5	96
66	5.0	1.6	70.5	77.1	79.5	97
67	6.0	1.4	78.5	85.9	91.0	94
68	6.4	1.6	75.0	83.0	91.0	92
69	6.9	1.8	80.0	88.7	91.0	98
70	6.6	1.8	74.5	82.9	90.5	92
73	6.7	1.8	76.5	85.0	91.0	94
74	6.8	1.3	84.0	91.1	91.0	100
75	6.1	1.6	76.0	83.7	91.0	92
76	6.0	2.1	79.0	87.1	91.0	96
77	6.9	4.9	79.5	91.3	91.0	100
80	5.1	3.4	78.0	86.5	91.0	95
81	5.2	5.4	74.5	85.1	91.0	94
				Average		94

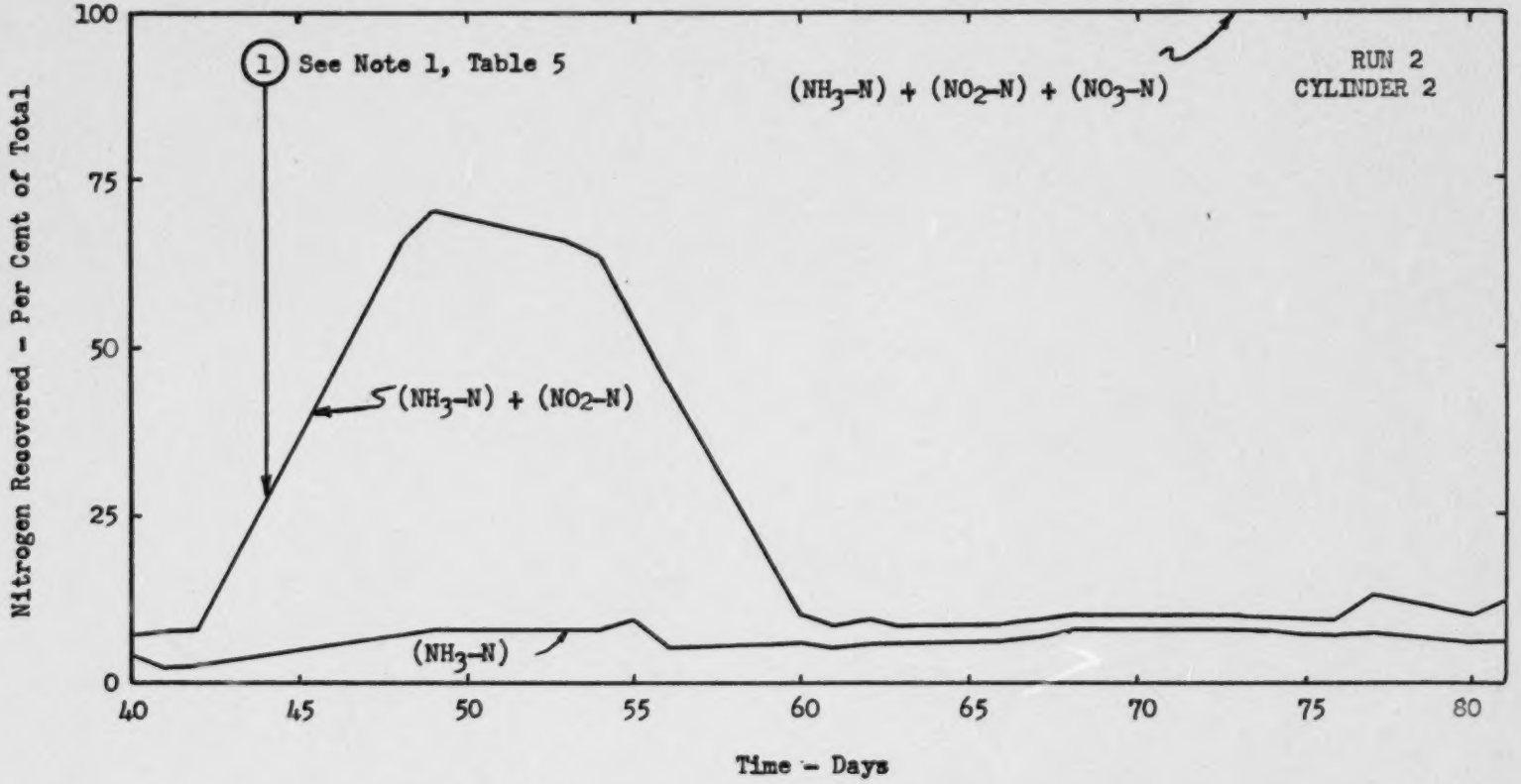

FIGURE 9 - DISTRIBUTION OF RECOVERED NITROGEN

TABLE 10

NITROGEN RECOVERY DATA

Run 2, Cylinder 3

Day	NH_3-N mg/l	NO_2-N mg/l	NO_3-N mg/l	Inorganic-N mg/l	CN-N fed mg/l	N-Recovery mg/l
40	8.6	10.5	48.5	67.6	63.0	103
41	8.3	9.9	47.0	65.2	63.0	102
42	9.4	7.9	51.0	68.3	63.0	104
46	9.4	12.8	41.0	63.2	63.0	100
47	8.6	8.6	50.0	67.2	63.0	103
48	9.0	3.4	53.2	65.6	63.0	102
49	9.3	0.8	60.0	70.1	63.0	111
52	8.0	3.8	55.5	67.3	63.0	106
53	7.7	4.6	33.0	45.3	63.2	72
54	7.1	3.6	28.5	39.2	63.2	62
55	7.8	3.4	46.5	57.7	63.2	91
56	6.9	2.6	56.0	64.5	63.2	102
59	6.9	2.9	58.0	67.8	63.2	107
60	6.8	2.4	49.0	58.2	60.7	96
61	6.0	2.9	51.0	59.9	60.7	99
62	7.2	4.6	46.5	58.3	60.7	96
63	7.1	7.3	52.2	66.9	60.7	110
66	7.0	12.4	41.0	60.4	60.7	100
67	6.7	13.7	45.0	65.4	60.7	108
68	6.7	14.4	44.5	65.6	60.7	108
69	7.0	16.0	45.5	68.5	60.7	113
70	6.1	19.4	41.0	66.5	60.7	110
73	7.1	22.8	30.0	59.9	64.5	93
74	7.0	27.0	30.0	64.0	61.6	104
75	6.1	25.3	28.5	59.9	64.5	93
76	6.0	31.0	28.5	65.5	64.5	102
77	7.6	36.6	28.5	72.7	64.5	112
80	6.4	36.8	24.0	67.2	64.5	104
81	7.0	36.8	22.0	64.8	64.5	100
				Average		100

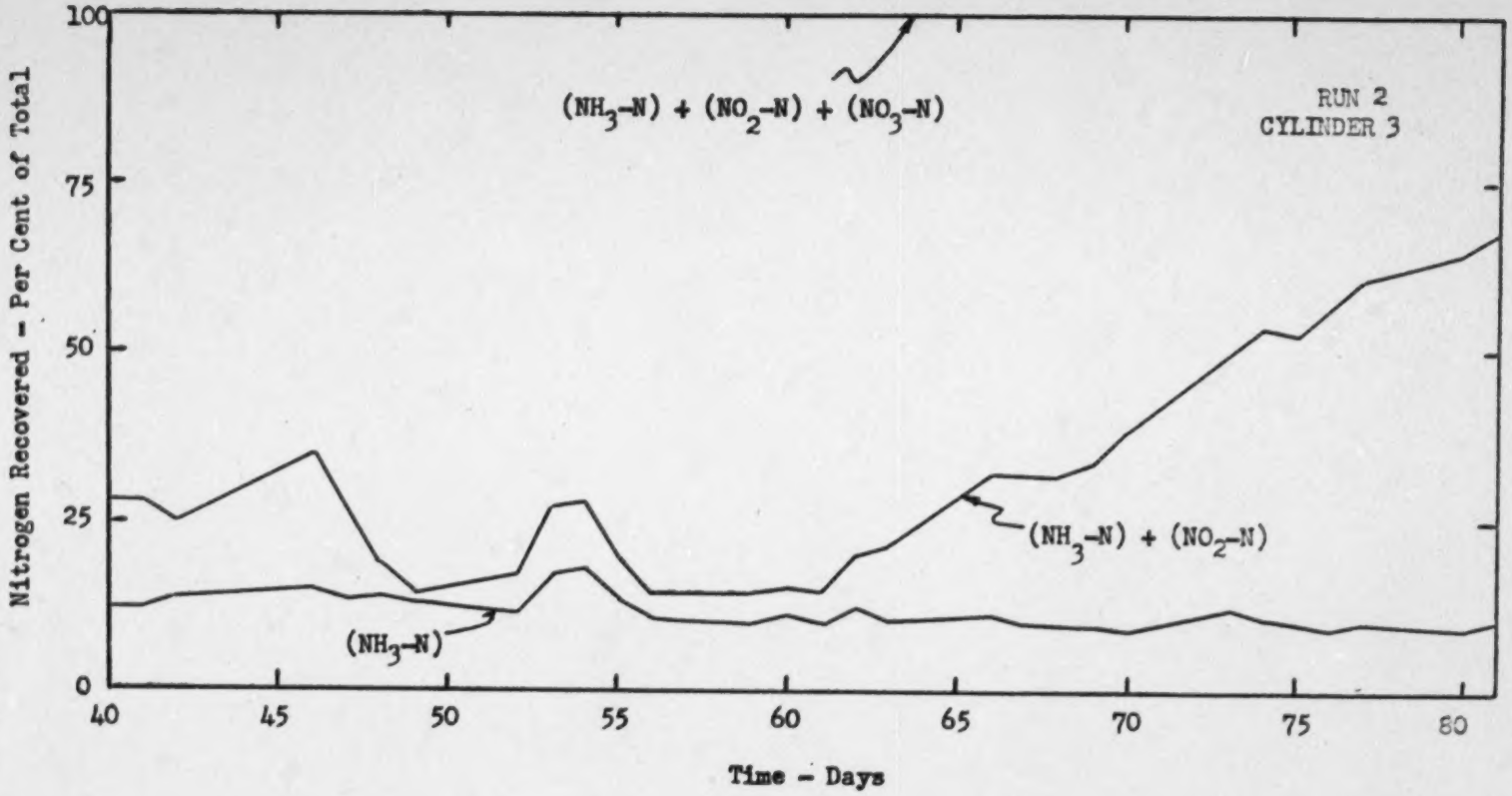

FIGURE 10 - DISTRIBUTION OF RECOVERED NITROGEN

TABLE 11

ORGANIC NITROGEN DATA

Run 2

Day	Cylinder No. 2					Cylinder No. 3				
	Susp. Solids mg/l	Vol. Solids mg/l	Org-N mg/l	N/ss %	N/vs %	Susp. Solids mg/l	Vol. Solids mg/l	Org-N mg/l	N/ss %	N/vs %
39	620	366	--	--	--	266	144	19.0	7.2	13.2
49	636	540	--	--	--	304	278	25.7	9.2	8.4
56	394	315	35.1	8.9	10.0	170	143	25.6	15.1	17.9
63	386	336	35.7	9.2	10.6	294	270	25.0	8.5	9.3
67	578	426	35.8	6.2	8.4	246	225	28.8	11.7	12.8
70	440	422	34.9	8.0	8.3	228	202	26.0	11.4	12.9
74	422	378	36.2	8.6	9.6	250	222	23.6	9.5	10.6
77	586	420	36.1	6.2	8.6	274	256	24.2	8.8	9.5
81	444	386	38.9	8.8	10.1	220	202	23.6	10.7	11.7
		Average		8.0	9.4		Average		10.2	11.8

which lasted from the 44th to 60th days. In Cylinder 3 the percentage of effluent nitrogen recovered as nitrite steadily increased during the latter half of the run; however, no residual cyanide was detected in the Cylinder at any time during the run.

The results of the mixed liquor organic nitrogen tests are presented in Table 11 along with the coincident results of the suspended solids and volatile solids tests. These results also varied somewhat but show that the percentage nitrogen by weight in the suspended solids averaged 8.0 and 10.2 in Cylinders 2 and 3 respectively and that the percentage nitrogen by weight in the volatile solids averaged 9.4 and 11.8 in Cylinders 2 and 3 respectively.

Nitrogen recovery and organic nitrogen data for Run 3 are presented in Tables 12, 13, and 14.

These data show that for Cylinder No. 3 (Table 13) in which cyanide served as the only source of carbon and nitrogen for the organisms an average of 98 percent of the added nitrogen was recovered in the effluent as ammonia (22.2 percent), nitrite (48.0 percent), or nitrate (29.8 percent). The organic nitrogen in this cylinder (Table 14) averaged 6.8 and 6.9 percent of the mixed liquor suspended and volatile solids respectively.

For Cylinder No. 2, in which the feed carbon was supplemented with sodium acetate from the 5th day of the run (Table 12) the percentage recovery of nitrogen varied

TABLE 12

NITROGEN RECOVERY DATA

Run 3, Cylinder No. 2

Day	Effluent NH_3-N mg/l	Effluent NO_2-N mg/l	Effluent NO_3-N mg/l	Effluent Sol. Org-N mg/l	Mix. Liquor Total Org-N mg/l	Est. Gain Total Org-N mg/l/day	Total N Recovered mg/l	CN-N fed mg/l	N Recovered percent(4)
2	6.9	16.5	17.5	--	--	--	40.9	61.8	66
3	6.0	22.2	19.5	--	19.8	--	47.7	61.8	77
6 (3)	6.2	22.2	22.5	7.0(2)	--	6.0	63.9(2)	61.8	103(2)
7	6.2	20.0	23.0	7.0(2)	--	6.0	62.2(2)	61.8	101(2)
8	0.1	20.0	18.3	7.0(2)	--	6.0	51.4(2)	61.8	83(2)
9	0.2	10.9	16.2	7.0(2)	--	6.0	40.3(2)	61.8	65(2)
10	0.9	1.0	2.5	7.0(2)	53.5	6.0	17.4(2)	63.2	28(2)
13	2.0	3.0	7.0	7.0(2)	--	6.0	25.0(2)	63.8	40(2)
14	1.3	4.6	12.5	7.3	--	6.0	31.7	63.8	48
15	1.4	7.0	17.3	9.1	--	6.0	40.8	63.8	64
16	0.4	30.0	22.0	7.8	--	6.0	66.2	63.8	104
17	0.2	23.7	24.5	6.6	88.3	6.0	61.0	63.8	96
20	0.1	29.0	24.0	--	113.4	6.0	59.1	63.8	93
22(1)	0.2	28.3	25.5	5.0	114.6	--	59.0	61.9	95
23	0.2	11.0	19.0	5.4	74.5	--	35.6	61.9	58(1)
24	0.5	21.5	21.0	4.0	94.0	--	47.0	61.9	76(1)

(1) Mechanical failure in water bath. Temperature 34°C. for 12 hours on the 22nd day.
(2) Estimated.
(3) Sodium acetate added on the 5th day.
(4) See Appendix A.

TABLE 13

NITROGEN RECOVERY DATA

Run 3, Cylinder No. 3

Day	Effluent NH_3-N mg/l	Effluent NO_2-N mg/l	Effluent NO_3-N mg/l	Total N Recovered mg/l	CN-N fed mg/l	N Recovered percent(1)
2	12.3	16.0	19.2	47.5	61.8	77
3	10.5	44.8	16.7	72.0	61.8	116
6	9.5	33.0	21.0	63.5	64.8	98
7	10.6	34.2	19.5	64.3	66.8	96
8	9.5	32.8	16.8	59.3	66.8	89
9	13.8	26.7	15.0	55.5	64.4	86
10	10.6	34.3	16.8	60.7	64.4	94
13	14.7	30.6	20.0	65.3	64.4	101
14	14.5	34.5	16.8	65.8	64.4	102
15	15.2	35.2	19.8	70.2	64.4	109
16	15.6	31.5	19.5	66.6	64.4	103
17	16.0	33.0	18.5	67.5	64.4	105
20	16.8	30.8	19.8	67.4	65.9	102
22	17.8	23.3	21.5	62.6	65.9	95
23	17.6	25.0	17.0	59.6	65.9	91
24	19.0	22.0	22.5	63.5	65.9	96
Average	14.0	30.3	18.8			98
% of Total	22.2	48.0	29.8			

(1) See Appendix A.

TABLE 13

NITROGEN RECOVERY DATA

Run 3, Cylinder No. 3

Day	Effluent NH_3-N mg/l	Effluent NO_2-N mg/l	Effluent NO_3-N mg/l	Total N Recovered mg/l	CN-N fed mg/l	N Recovered percent(1)
2	12.3	16.0	19.2	47.5	61.8	77
3	10.5	44.8	16.7	72.0	61.8	116
6	9.5	33.0	21.0	63.5	64.8	98
7	10.6	34.2	19.5	64.3	66.8	96
8	9.5	32.8	16.8	59.3	66.8	89
9	13.8	26.7	15.0	55.5	64.4	86
10	10.6	34.3	16.8	60.7	64.4	94
13	14.7	30.6	20.0	65.3	64.4	101
14	14.5	34.5	16.8	65.8	64.4	102
15	15.2	35.2	19.8	70.2	64.4	109
16	15.6	31.5	19.5	66.6	64.4	103
17	16.0	33.0	18.5	67.5	64.4	105
20	16.8	30.8	19.8	67.4	65.9	102
22	17.8	23.3	21.5	62.6	65.9	95
23	17.6	25.0	17.0	59.6	65.9	91
24	19.0	22.0	22.5	63.5	65.9	96
Average	14.0	30.3	18.8			98
% of Total	22.2	48.0	29.8			

(1) See Appendix A.

TABLE 14

ORGANIC NITROGEN DATA

Run 3

Day	Cylinder No. 2					Cylinder No. 3				
	Susp. Solids mg/l	Vol. Solids mg/l	Org-N mg/l	N/ss %	N/vs %	Susp. Solids mg/l	Vol. Solids mg/l	Org-N mg/l	N/ss %	N/vs %
3	--	--	19.8	--	--	--	--	16.1	--	--
10	506	--	53.5	10.6	--	230	--	17.8	7.7	--
17	816	780	88.3	10.8	11.3	--	--	--	--	--
20	1040	980	113.4	10.9	11.6	258	240	17.6	6.8	7.3
22(1)	1240	1162	114.6	9.2	9.9	--	--	--	--	--
23	1216	1166	74.5	6.1	6.4	--	--	--	--	--
24	1262	1120	95.0	7.5	8.5	268	236	15.4	5.8	6.5
Average				9.2	9.6				6.8	6.9

(1) Mechanical failure in water bath. Temperature 34°C. for 12 hours on 22nd day.

considerably. On the 6th and 7th days the estimated recovery was approximately 100 percent with 70 percent of the total recovered as nitrite and nitrate. From the 7th to the 10th days the recoveries decreased to a low of 28 percent and then increased to average 97 percent during the period from the 16th to the 22nd day. In this last period the recovered nitrogen averaged 84 percent nitrite plus nitrate, 1 percent ammonia, 8 percent soluble organic nitrogen and 7 percent cell nitrogen. The organic nitrogen expressed as a percentage of the volatile solids (Table 14) varied from 11.6 to 6.4 and averaged 9.6 during the run.

Carbon

Carbon recovery tests were made only during Run 1. Table 15 gives this data for a 13 day period for two, 3-liter cylinders being fed 117 mg CN^-/day and 57 mg CN^-/day respectively. The cylinders were similar to those used in the initial 185 day run; however, the data were collected in a subsequent run.

The table shows that approximately 98% of the carbon added as cyanide was recovered as carbon dioxide in the effluent air from both cylinders.

TABLE 15

CARBON RECOVERY DATA

Run 1

Day	Cylinder No. 1 (1) Carbon Recovered mg.	Cylinder No. 1 (1) Carbon Added mg.	Cylinder No. 3 (2) Carbon Recovered mg.	Cylinder No. 3 (2) Carbon Added mg.
1	55.5	51.4	23.3	25.1
2	59.3	49.2	20.2	25.1
3	52.3	51.4	32.6	25.1
4	48.2	51.4	22.0	24.0
5	50.0	51.4	23.7	25.1
6	39.0	49.2	24.0	24.0
7	50.8	51.4	24.0	25.1
8	47.8	49.2	23.4	22.9
9	49.2	51.4	25.0	25.1
10	49.0	47.0	25.5	25.1
11	42.4	51.4	24.2	24.0
12	48.8	49.2	21.6	25.1
13	49.0	51.4	--	--
Total	641.3	655.0	289.5	295.7
% Carbon Recovery - 97.91			% Carbon Recovery - 97.90	

(1) Feed = 117 mg CN^-/day at hourly intervals.
(2) Feed = 57 mg CN^-/day at hourly intervals.

Continuous Operation

The results from the 31 days of operation of the continuous feed pilot plant are presented in Table 16. They show that at the lower cyanide feed concentrations all of the cyanide was removed, while at the higher concentrations a varying percentage removal was obtained. For the most part the percentage removal was lowest immediately following an increase in cyanide feed concentration after which it slowly increased to a value between 80 and 90 percent.

TABLE 16

CONTINUOUS FEED DATA

Day	CN^- Feed mg/day	CN^- Feed Equiv. mg/l	Effluent CN^- mg/l	CN^- Removal percent
1	240	0.44	--	100
2	240	0.44	--	100
3	300	0.55	--	100
4	300	0.55	--	100
5	300	0.55	--	100
6	500	0.92	--	100
7	600	1.10	--	100
8	750	1.38	--	100
9	1000	1.83	--	100
10	1500	2.75	--	100
11	2000	3.67	--	100
12	3000	5.50	--	100
13	4000	7.35	5.9	20
14	4000	7.35	5.5	25
15	--	--	--	--
16	2000	3.67	0.5	86
17	2000	3.67	0.3	92
18	2000	3.67	--	100
19	2000	3.67	--	100
20	2000	3.67	0.7	81
21	2000	3.67	0.3	92
22	2000	3.67	0.7	81
23	2000	3.67	0.7	81
24	2000	3.67	--	--
25	4000	7.35	5.0	32
26	4000	7.35	4.6	37
27	4000	7.35	3.2	56
28	4000	7.35	1.5	80
29	4000	7.35	1.2	84
30	4000	7.35	2.2	70
31	4000	7.35	1.0	86

IV. DISCUSSION

The results reported under "Residual Cyanide" (Table 1) indicate that, when the systems were not upset by a mechanical failure, 99+ percent of the cyanide fed to the systems was metabolized by the organisms. This fact was corroborated by the carbon recovery data which indicated that 97.9 percent of the cyanide carbon fed to either system was recovered as carbon dioxide.

The results reported under "Mixed Liquor Suspended Solids" for batch operation (Figures 6 and 7) indicate that, when the cyanide radical served as the only source of carbon and nitrogen for the organisms, the determination of an equilibrium weight of mixed liquor suspended solids for specific cyanide feed rates was inconclusive. In Run 1 at about the 115th day of operation, the suspended solids concentration appeared to reach an equilibrium at 220 mg/l and 110 mg/l in the cylinders being fed 120 mg CN^-/day (Cylinder No. 2) and 60 mg CN^-/day (Cylinder No. 3) respectively; however, mechanical failures make these data somewhat questionable except for an order of magnitude value. During the last 19 days of operation (days 166 to 185) when no mechanical failures occurred, the solids concentrations completely reversed and appeared to reach equilibriums at 150 mg/l and 220 mg/l in Cylinders No. 2 and No. 3 respectively.

A conclusive explanation for this shift in solids

concentration cannot be given with the data available at this time; however, these data do indicate a different biological composition in the two systems which could account for the unusual results.

The nitrogen recovery data (Tables 6 and 7) indicate that from the 131st to the 169th days considerable change occurred in the distribution of nitrogen in the supernatant of Cylinder No. 3 while little change occurred in Cylinder No. 2. This change in Cylinder No. 3 involved a decrease in the percentage nitrites from 50.0 to 20.7 and an increase in the percentage nitrates from 35.3 to 76.4. During this same period in Cylinder No. 2 the percentage nitrites decreased only slightly from 70.0 to 67.0 and the percentage nitrates increased only slightly from 15.8 to 23.9. This change in nitrogen distribution and the high percentage nitrates in the supernatant of Cylinder No. 3 indicates the growth, during this period, of a nitrifying organism such as nitrobacter, which is known to convert nitrites to nitrates and to have little effect upon other forms of nitrogen (20). The additional growth of such an organism in Cylinder No. 3 could have provided the additional suspended solids. This organism could also have been present in Cylinder No. 2, but its growth may have been inhibited either by the higher cyanide feed in this cylinder or the high concentration of nitrites that existed throughout the experiment, or by both, for the latter is known to be a bacterial inhibitor (3).

Additional evidence of a different biological composition in the two systems is the higher percentage of nitrogen in the suspended solids of Cylinder No. 2 (Table 8).

At the end of Run 2 the suspended solids concentrations (Figure 7) appeared to reach equilibriums of 480 mg/l in the cylinder being fed 175 mg CN^-/day (Cylinder No. 2) and 240 mg/l in the cylinder being fed 120 mg CN^-/day (Cylinder No. 3). Thus a feed ratio of approximately 1.45/1 produced a suspended solids ratio of approximately 2/1.

This difference in ratios also might have been caused by the difference in biological composition of the two systems previously described. Figures 9 and 10 indicate that, except for a period from the 44th to the 60th days in Cylinder No. 2, the effluent from Cylinder No. 2 contained a higher percentage of nitrate and a lower percentage of nitrite than did the effluent from Cylinder No. 3. Thus again the additional growth of a nitrifying organism could have provided the additional suspended solids in Cylinder No. 2. Evidence of this different biological system is again corroborated by the higher percentage of nitrogen in the suspended and volatile solids in the cylinder having the higher concentration of nitrites in the effluent (Cylinder No. 3).

In Run 3, 48 percent of the effluent nitrogen in the control cylinder (No. 3) was recovered as nitrite (Table 13) and the mixed liquor suspended solids averaged about 250 mg/l

a concentration quite similar to that obtained in the corresponding cylinder in Run 2 (No. 3). In the sodium acetate supplemented cylinder (No. 2), the organisms were rapidly acclimated to the acetate and within eleven days the 95 percent reduction in COD (Table 6) indicated that most of the acetate was being metabolized. This metabolism verifies the findings of Trelawney, et al (21) who reported that organisms acclimated to a cyanoacetate media grew very well on an acetate media.

From the results of Run 3, Cylinder 2 (Figure 8), an equilibrium weight of mixed liquor suspended solids for the particular cyanide-acetate feed rate could not be determined as the run had to be terminated before an equilibrium was established. However, at the end of the run a comparison of the suspended solids in the two cylinders indicates that approximately 80 percent of the suspended solids in Cylinder No. 2 resulted from the additional acetate in the feed. Thus very little growth was realized from the cyanide in the feed and any equilibrium weight would depend largely upon the amount and character of the organic matter fed with the cyanide.

This lack of growth in cultures fed cyanide as their only source of carbon and nitrogen was also noted by Trelawny, et al (21) and Ettinger (4); however, although little growth appears, the successful operation during Runs 1 and 2 indicates that the metabolism in such a system is sufficient to maintain it.

The addition of sodium acetate to the cyanide feed in the system also appeared to decrease the sensitivity of the system to some changes in its environment. In Run 3 the acetate supplemented cylinder operated successfully at pH values as high as 9.5. In non-supplemented cylinders in any of the runs such a high pH was always indicative of a high residual cyanide in the cylinder.

The results reported under "Nitrogen" (Tables 6, 7, 9, 10, 13) show that in nearly all the cylinders in which cyanide served as the only source of carbon and nitrogen, 90 to 100 percent of the cyanide nitrogen was recovered in the effluent as ammonia, nitrite or nitrate nitrogen. The missing nitrogen in these cylinders and in the cylinders in which less than 90 percent of the nitrogen was recovered (Cylinder No. 2, Run 1 and Cylinder 3, latter part of Run 1) has not been accounted for. Tests indicated that it was not ammonia or cyanide-nitrogen blown from the system during the aeration process. It could possibly have been respired as nitrogen gas or converted to soluble organic nitrogen but no tests were made for these forms of nitrogen in these cylinders. In any case, it is only a very small portion of the nitrogen added to the system.

In the acetate supplemented cylinder, cyanide nitrogen was recovered as ammonia, nitrite, nitrate and soluble organic nitrogen in the effluent and organic nitrogen in the mixed liquor suspended solids (Table 12). These sources accounted for 97 percent or more of the cyanide nitrogen

during the first three days the acetate was added to the system and after the 16th day when approximately 95 percent of the COD was being removed by the system. Between these times the nitrogen recovery sagged to a low of 28 percent on the 10th day. This missing nitrogen cannot be accounted for from the data collected; however, it is possible that it was ammonia blown from the system because of its high pH (about 9.0).

The decrease in the interval between feeds from one hour to one-half hour to a continuous feed appeared to have little effect on the ability of the system to metabolize the cyanide. The continuous feed run was a preliminary one and the results were limited; however, they do indicate that such a system could be successfully operated.

V. CONCLUSIONS

From the information gained in these studies it can be concluded that:

1. Potassium cyanide can be metabolized and 99+ percent of it removed from solution by an activated sludge culture using cyanide, fed at one hour and one-half hour intervals, as the only source of carbon and nitrogen.
2. Suspended solids can be maintained in a cyanide acclimated system without additional sewage feed.
3. From 75 to 100 percent of the cyanide nitrogen is converted to ammonia, nitrite, and nitrate in a cyanide acclimated system.
4. In a cyanide acclimated system 97.9 percent of the feed cyanide-carbon is converted to carbon dioxide.
5. Sodium acetate can be metabolized by an activated sludge culture previously acclimated to cyanide as its only source of carbon and nitrogen.
6. A cyanide-acetate acclimated culture will remove 95 percent of the COD and 99+ percent of the cyanide from an influent solution feeding 1780 mg COD/day and 120 mg CN^-/day.
7. In a cyanide-acetate acclimated system from 28 to 100 percent of the influent nitrogen can be recovered as cell growth and ammonia, nitrite,

nitrate, or soluble organic nitrogen in the effluent.

8. The continuous feeding of potassium cyanide does not inhibit its ability to metabolize and remove cyanide from solution.

VI. RECOMMENDATIONS FOR FUTURE STUDY

The following suggestions for future study on the aerobic metabolism of cyanogenic compounds should be of value in determining the feasibility of aerobic biological treatment for wastes containing cyanide:

1. Continue the studies on a continuous flow activated sludge system. In this study particular emphasis should be placed on a cyanide feed supplemented with known organic compounds, cyanides complexed with heavy metal ions, and shock loads on the system.
2. A thorough bacteriological study of the micro-organisms involved in the metabolism.
3. A study to determine whether the metabolism involved is a direct metabolism of the cyanogenic compound or a metabolism of the end products resulting from hydrolysis of the compound.

The above suggestions have resulted directly from the research reported herein and from discussions with various members of the staff of The Pennsylvania State University.

VII. PUBLICATIONS

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APPENDIX A

Sample Calculation for Nitrogen Recovery - Run 1

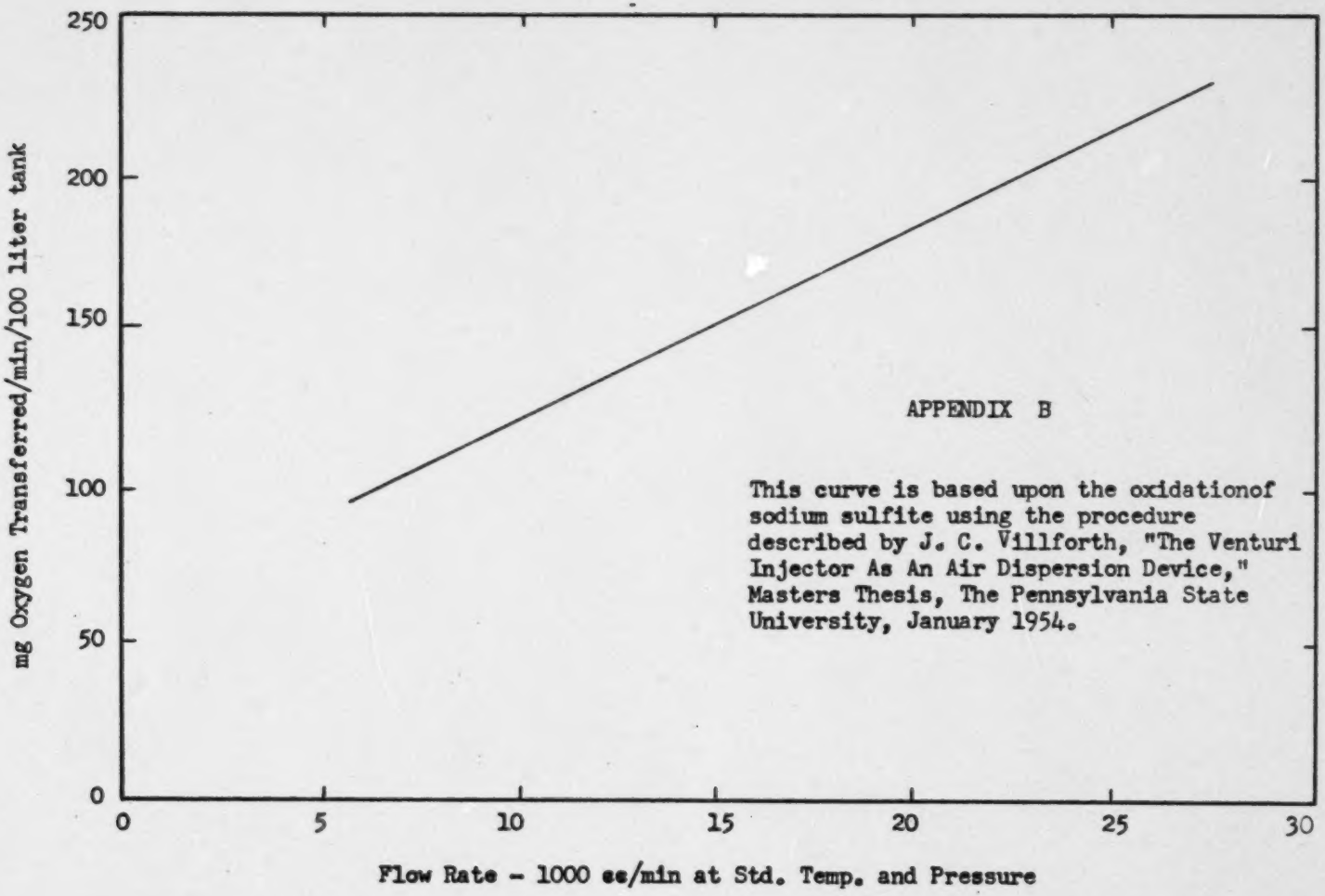
Use values in Table 4, Cylinder 2, days 138 and 139, The total volume of Cylinder No. 2 at the end of 24 hours was 3.14 liters.

	Day 138	Day 139
NH_3	4.5 mg/l	4.6 mg/l
NO_2	33.5 mg/l	32.0 mg/l
NO_3	7.2 mg/l	7.4 mg/l
Total N =	45.2 mg/l	44.0 mg/l

Since one liter was withdrawn daily, 2.14 x 45.2 = 96.6 mg N remaining at the end of the 138th day. Therefore, (44.0 x 3.14) - 96.6 = 41.4 mg of nitrogen gained from the 138th to the 139th day in Cylinder No. 2

Sample Calculation for Nitrogen Recovery - Run 2, 3

One hundred percent nitrogen recovery was realized when the nitrogen added to the cell per day and the nitrogen content of the one-liter of effluent withdrawn per day equalled the cyanide nitrogen added per day.



APPENDIX B

This curve is based upon the oxidationof sodium sulfite using the procedure described by J. C. Villforth, "The Venturi Injector As An Air Dispersion Device," Masters Thesis, The Pennsylvania State University, January 1954.

FIGURE 11 -.OXYGEN TRANSFER CURVE